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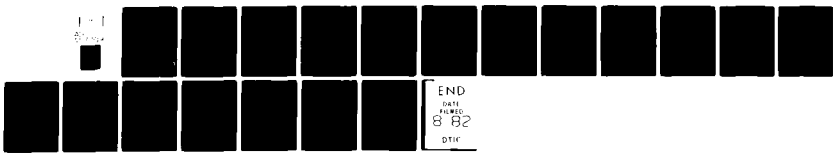

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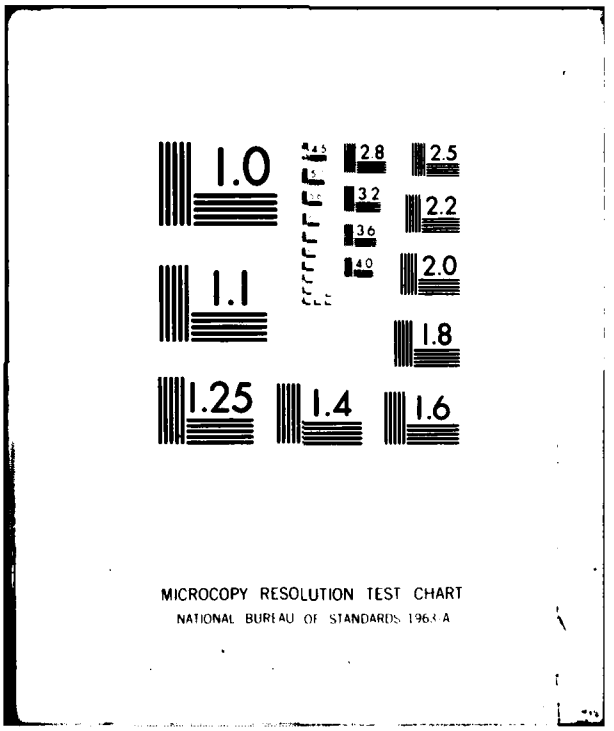
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ANNUAL PROGRESS REPORT NO. 1

The Etiology and Pathogenesis of Viral Gastroenteritis

by

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July 31, 1982

SUMMARY

The purpose of this project is to identify, cultivate, and characterize etiologic agents of viral gastroenteritis of man and to study the pathogenesis of infection in order to provide information necessary to attain the ultimate goals of prevention and cure of this common syndrome. Progress achieved during the current year can be summarized as follows. Monoclonal antibody techniques have been utilized successfully to prepare purified antibodies against Norwalk virus with 5 positive clones now prepared. These monoclonal antibodies are being tested as probes to detect a potential group specific antigen common to the noncytopathic Norwalk-like virus group and to recognize these viruses in nature. In addition, studies are underway to try to cultivate Norwalk virus, using methods that proved successful for the in vitro cultivation of hepatitis A virus and rotavirus.

We have continued studies on the etiology and epidemiology of gastroenteritis with data acquired in 5 study populations: An accelerated development of age specific prevalence of antibody to Norwalk virus has been found in inhabitants of a rural Thailand village. Two of 62 American Peace Corps volunteers were infected with Norwalk virus during their early stay in Thailand. The epidemiology of two nursing home outbreaks of Norwalk virus diarrhea has been studied. This virus has also been shown to be the likely cause of at least a small proportion of endemic family diarrhea in Texas. Finally, both Norwalk virus and rotavirus were shown to play roles in diarrhea among adult travelers to Mexico.

The IgM antibody response to Norwalk virus was assessed in fractionated serum samples from volunteers challenged and rechallenged with the virus. An IgM response occurred in ill subjects, whether or not preexisting total serum antibody was present. The peak response occurred about 2 weeks after illness but IgM was detectable in lower titers for up to 21 weeks after infection. Upon longterm exposure to the virus, previously ill volunteers who had produced IgM antibody once again mounted a secondary IgM response with recurrent illness, which was greater than the first IgM response. Thus, the IgM response is not restricted to primary infection. The potential diagnostic utility of IgM testing for Norwalk virus should be assessed from studies of naturally occurring disease outbreaks.

CONTENTS

BACKGROUND INFORMATION ON VIRAL GASTROENTERITIS

This research program is designed (a) to identify, cultivate, and characterize etiologic agents of viral gastroenteritis, and (b) to study the pathogenesis of infection in order to provide information necessary to achieve goals of disease prevention and cure.

Acute viral gastroenteritis is an extremely common illness that affects all age groups and occurs in both epidemic and endemic forms (1). It is second in frequency only to the common cold among illnesses affecting United States families under epidemiological surveillance. It is also responsible for some of the common travelers' diarrhea encountered in Latin America, Africa, and Asia. The illness varies in its clinical presentation, but in general it is self limited, begins with an explosive onset, and consists of varying combinations of diarrhea, nausea, vomiting, low grade fever, abdominal cramps, headache, anorexia, myalgia and malaise. It can be severe, indeed fatal, in the elderly, infant, debilitated or malnourished patient.

Viral gastroenteritis occurs primarily in two epidemiologically distinct clinical forms (1). One entity is characteristically epidemic and is responsible for family and community-wide outbreaks of gastroenteritis among older children and adults. The older medical literature gives a variety of descriptive labels to this one to two day illness, such as winter vomiting disease, epidemic collapse, viral diarrhea, epidemic diarrhea and vomiting, and acute infectious nonbacterial gastroenteritis. In recent years, a newly discovered agent, Norwalk virus, has been shown to be responsible for about one-third of these disease outbreaks in the United States. Other Norwalk-like viruses have also been discovered such as Hawaii agent, and although they have not yet been studied epidemiologically, they are likely to be responsible for many more epidemic cases of this illness.

The second clinical entity is usually sporadic and occasionally epidemic and it occurs predominantly in infants and young children (1). However, as noted below, it can occur in adults. This form of illness typically produces severe diarrhea that commonly lasts for five to eight days and is usually accompanied by fever and vomiting. Rotavirus, which was discovered during the 1970's, is responsible for approximately one half of the cases of this clinical entity requiring hospitalization. Although the major target of rotavirus is the very young, it can produce surprising severe clinical disease in adults (1).

Despite the frequency of viral gastroenteritis syndromes, the etiology of these illnesses remained obscure until the 1970's. The principal investigator began his studies into the etiology and pathogenesis of this illness in 1970, with the initial goal of development of materials and methodology necessary for an understanding of this disease. Initially, he transmitted enteritis to healthy adult volunteers by the oral administration of bacteria-free, toxin-free stool filtrates derived from several outbreaks of the disease. These studies led to the discovery of the first major group

1. Blacklow, N.R. and Cukor, G.: Viral gastroenteritis. New Eng. J. Med. 304:397-406,1981.

of agents responsible for viral diarrhea, the Norwalk-like viruses (2). The prototype Norwalk virus, which is still currently noncytopathic in vitro and not disease producing for experimental animals, was initially described by the investigator and colleagues as a small lipid-free virus (3). It was later visualized in infectious stool filtrates and partially characterized by immune electron microscopy (IEM) and ultracentrifugation (4). Other similar Norwalk-like viruses, such as Hawaii and Ditchling viruses, have been uncovered by similar techniques; these two agents appear to form (with Norwalk virus) three immunologically distinct agents based on IEM studies (1).

The investigators have shown that the Norwalk and Hawaii agents both produce a mucosal lesion of the proximal human small intestine, the likely site for replication of these viruses (5,6). This lesion is accompanied by transient small intestinal malabsorption, and also by delayed gastric emptying despite normal gastric morphology and secretory function (7).

The investigator has also established that clinical immunity to Norwalk virus is novel and fails to fit immunologic concepts traditionally associated with common human viral illnesses (8): pre-existing serum antibody is paradoxically associated with the development of illness in volunteers, and lack of pre-challenge antibody is found in volunteers who remain well after expo-

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2. Dolin, R., Blacklow, N.R., DuPont, H. et al: Transmission of acute infectious nonbacterial gastroenteritis by oral administration of stool filtrates. J.Infect.Dis. 123:307-312,1971.

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4. Kapikian, A.Z., Wyatt, R.G., Dolin, R. et al: Visualization by immune electron microscopy of a 27nm particle associated with acute infectious nonbacterial gastroenteritis. J.Virol. 10:1075-1081,1972.

5. Schreiber, D.S., Blacklow, N.R.,Trier,J.S.: The mucosal lesion of the proximal small intestine in acute infectious nonbacterial gastroenteritis. New Eng. J.Med. 288:1318-1323,1973.

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8. Parrino,T.A.,Schreiber,D.S.,Trier,J.S.,Kapikian,A.Z.,Blacklow,N.R.: Clinical immunity in acute gastroenteritis caused by Norwalk agent. New Eng.J.Med. 297:86-89,1977.

sure to the virus and also fail to seroconvert to the agent(8-10). In addition, antibody to Norwalk virus in pre-challenge intestinal fluids has been found predominantly in those volunteers who subsequently developed illness. At least 2 forms of clinical immunity exist for Norwalk virus: one group of subjects (persistently lacking antibody) maintains long-term immunity to the virus as shown by lack of illness after initial challenge and after rechallenge up to 34 months later. A second group of volunteers (persistently possessing antibody) is susceptible to infection both upon initial exposure and again upon rechallenge 27 to 42 months later. Short term immunity exists to the virus when ill subjects are reexposed after 6 to 14 weeks.

During the past 4 years, investigators at the National Institutes of Health, as well as the principal investigator, have developed a radioimmunoassay (RIA) technique for the detection of Norwalk virus in diarrheal stools and for quantitation of antibody to the agent (9,10). The RIA represents a major advance in the study of this virus, and now provides a laboratory handle for studies to cultivate the virus in vitro. The principal investigator has already used the RIA to study forms of clinical immunity to Norwalk virus (10) (also see the preceding paragraph), and to show that Norwalk RIA serum antibody prevalence levels rise during adolescence in the U.S. (10). It has also been observed that antibody to Norwalk virus is acquired at a significantly earlier age in less developed and tropical areas than in more developed and nontropical areas (11,12). The RIA test has also been used to show that Norwalk virus is responsible for approximately one-third of viral gastroenteritis epidemics that occur in the United States (13).

It should be noted that the RIA test for Norwalk virus and its antibody is currently available in only a few research laboratories including that of the principal investigator. This is because the procedure requires the use of precious limited human volunteer materials (stools and sera). The Norwalk RIA procedure for the first time permits the large-scale rapid testing of stool and serum specimens from individuals for evidence of infection with Norwalk virus. Such studies have already shown the epidemiologic importance of Norwalk

8. Parrino,T.A.,Schreiber,D.S.,Trier,J.S.,Kapikian,A.Z.,Blacklow,N.R.:Clinical immunity in acute gastroenteritis caused by Norwalk agent. New Eng. J. Med. 297:86-89,1977.

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10. Blacklow,N.R.,Cukor,G.,Bedigian,M.K. et al:Immune response and prevalence of antibody to Norwalk enteritis virus as determined by radioimmunoassay. J. Clin.Micro. 10:903-909,1979.

11. Greenberg,H.B.,Valdesuso,J.,Kapikian,A.Z.,et al: Prevalence of antibody to the Norwalk virus in various countries. Infect.&Immun. 26:270-273,1979.

12. Cukor,G.,Blacklow,N.R.,Echeverria,P.,Bedigian,M.K.,Puruggan,H. and Basaca-Sevilla, V.: Comparative study of the acquisition of antibody to Norwalk virus in pediatric populations. Inf. & Immunity 29:882-823,1980.

13. Greenberg,H.B.,Valdesuso,J.,Yolken,R.H. et al: Role of Norwalk virus in outbreaks of non-bacterial gastroenteritis. J.Inf.Dis. 139:564-568,1979.

virus in various parts of the world, including its involvement in waterborne foodborne, and shipborne outbreaks of acute gastroenteritis (13-17). In addition, the investigator and colleagues have shown Norwalk virus to be a cause of travelers' diarrhea in Mexico and Thailand (18,19).

During the past 7 years, a second viral enteric pathogen of man has been identified and is now known to be a major cause of diarrhea in young children (20,21). It can also produce illness in adults (22-24). This pathogen, rotavirus, has been identified by electron microscopy in stool filtrates derived from ill individuals (20,21). Serologic assay techniques have been developed

13. Greenberg, H.B., Valdesuso,J.,Yolken,R.H. et al: Role of Norwalk virus in outbreaks of non-bacterial gastroenteritis. J.Inf.Dis. 139:564-568,1979.

14. Taylor,J.W.,Gary,G.W. and Greenberg,H.B.: Norwalk-related gastroenteritis due to contaminated drinking water. Amer. J. Epid. 114:584-592,1981.

15. Koopman,J.S.,Eckert,E.A.,Greenberg,H.B. et al: Norwalk virus enteric illness acquired by swimming exposure. Amer.J.Epid. 115:173-177,1982.

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17. Griffin,M.R.,Surowiec,J.J.,McCloskey,D.I.,Capuano,B.,Pierzynski,B.,Quinn, M.,Wojnarski,R.,Parkin,W.E.,Greenberg,H. and Gary.G.W.: Foodborne Norwalk virus. Amer. J. Epid. 115:178-184,1982.

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19. Echeverria, P., Blacklow, N.R., Sanford, L.B. and Cukor, G.: Travelers' diarrhea among American Peace Corps volunteers in rural Thailand. J.Inf.Dis. 143:767-777, 1981.

20. Bishop, R.F., Davidson, G.P., Holmes, I.H., et al: Detection of a new virus by electron microscopy of fecal extracts from children with acute gastroenteritis. Lancet 1:149-151,1974.

21. Kapikian,A.Z.,Kim,H.W.,Wyatt,R.G. et al: Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. New Eng. J. Med. 294:965-972,1976.

22. vonBonsdorff,C.H.,Hovi,T.,Makela,P.: Rotavirus infections in adults in association with acute gastroenteritis. J.Med.Virol. 2:21,1978.

23. Bolivar,R.,Conklin,R.H.,Vollett,J.J. et al: Rotavirus in travelers' diarrhea: Study of an adult student population in Mexico. J.Inf.Dis. 137:324-327,1978.

24. Wenman,W.M.,Hinde,D., Feltham,S. et al: Rotavirus infection in adults:results of a prospective family study. New Eng.J.Med. 301:303-306,1979.

for this agent by our laboratory and others and can detect antibodies in human sera (25,26). In addition, rotavirus has been identified by our laboratory and others in diarrheal feces by RIA or enzyme-linked immunosorbent assay (ELISA) techniques (27-29). Laboratory techniques now permit in vitro study of the biologic properties of rotavirus. We have already used these methods to establish the role of this virus in diarrhea in several nations around the world, including travelers' diarrhea experienced by U.S. military populations overseas (18,30-34). Recently, Japanese scientists have successfully cultivated human rotavirus in cell culture by incorporating low concentrations of trypsin into the culture medium (35).

25. Kapikian,A.Z.,Kim,H.W.,Wyatt,R.G. et al: Reovirus-like agent in stools: association with infantile diarrhea and development of serologic tests. Science 185:1049-1053,1974.

26. Blacklow, N.R.,Echeverria,P.,Smith,D.H.: Serologic studies with reovirus-like enteritis agent. Infect.Imm. 13:1563-1566,1976.

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29. Blacklow, N.R. and Cukor, G.: Viral gastroenteritis agents. Manual of Clinical Microbiology, Third Edition, Ed. Lennette, E. et al (American Society for Microbiology) 891-898,1980.

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32. Echeverria, P., Hodge, F.A., Blacklow, N.R. et al: Travelers' diarrhea among United States Marines in South Korea. Amer. J. Epid., 108:68,1978.

33. Echeverria, P., Ramirez, G., Blacklow, N.R. et al: Travelers' diarrhea among United States Army troops in South Korea. J. Inf.Dis. 139:215,1979.

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35. Urasawa, T., Urasawa, S., and Taniguchi, K.: Sequential passages of human rotavirus in MA-104 cells. Microbiol. Immunol. 25:1025-1035,1981.

18. Kesswick,B.H.,Blacklow,N.R.,Cukor,G.,DuPont,H.L. and Vollet,J.J.: Norwalk virus and rotavirus in travellers' diarrhoea in Mexico.The Lancet 1:109-110,1982.

During the past few years, several other potential agents of viral gastroenteritis have been described, including enteric adenovirus,calicivirus, enteric coronavirus and astrovirus (1). The medical importance of these agents is currently not known, in contrast to Norwalk virus and rotavirus.

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The following Annual Progress Report covers only work accomplished during the period of August 1, 1981 - July 31, 1982.

IDENTIFICATION AND GROWTH OF NORWALK-LIKE VIRUSES

I. Monoclonal Antibody Studies With Norwalk Virus

Recently, 5 positive monoclonal antibody producing clones against Norwalk virus have been obtained in our laboratory. We have hyperimmunized mice with a partially purified Norwalk particle and RIA antigen-containing infectious human stool filtrate. Spleen cells from immunized mice have then been hybridized with SP2 mouse myeloma cells. Supernatant fluids from resultant hybridomas have then been screened in a Norwalk RIA in which human antibody to Norwalk virus serves as a coating reagent, followed by reaction with a Norwalk particle-containing stool (not the immunizing stool), followed by addition of the test hybridoma fluids, and followed lastly by labeled antimouse immunoglobulin as the detection antibody. One hybridoma is positive in this assay and appears to be Norwalk virus-specific since it is not reactive with extraneous stool antigens by a confirmatory RIA procedure. This confirmatory RIA procedure is the same as the outlined above, except that a pre-virus challenge human stool (Norwalk-RIA negative) from the same individual who provided the Norwalk-positive stool is substituted as the stool reagent in the RIA.

Four clones derived from the original hybridoma also continue to be stable and specific for Norwalk virus. They fail to react with a human serum lacking Norwalk antibody as a coating reagent in the RIA. All clones were derived by limiting dilution on a feeder cell layer. They all react with a panel of 5 known Norwalk positive stools, and fail to react with 6 known Norwalk negative control stools. Clones are being stored frozen as well as being expanded for further study. In addition, it should be noted that additional newly immunized animals are now under study. It is clear that we are now producing monoclonal antibodies against Norwalk virus, and a new area for laboratory investigation with Norwalk virus has arisen.

1. Blacklow, N.R. and Cukor, G.: Viral gastroenteritis, New Eng. J. Med. 304: 397-406,1981.

2. Viral Cultivation Studies

We are now using three cell lines which have proven useful for the cultivation of hepatitis A virus or rotavirus in order to attempt propagation of Norwalk virus. These simian cell lines are MA104, FrhK4, and FrhL2 (35-38). When replicate cultures were 90% confluent, they were inoculated with Norwalk virus and placed on medium containing 2% fetal calf serum. The supernatant fluids are now being checked weekly for the presence of Norwalk virus by radioimmunoassay (RIA), a method which as been used successfully for detection of hepatitis A virus growth in cell culture (39). Once a month a culture is being harvested by repeated freezing and thawing followed by sonication, and then tested for Norwalk virus by RIA. Our plans are to keep some inoculated cultures for several months prior to harvesting since this has been shown to be necessary from some studies that have grown hepatitis A. Our studies have been initiated very recently and thus far only a small amount of negative data have been obtained.

ETIOLOGY AND EPIDEMIOLOGY OF GASTROENTERITIS IN VARIOUS POPULATIONS

Because of the development of new laboratory techniques, including RIA, it has become possible in recent years for the first time to assess the role of newly discovered viral agents in outbreaks of infectious nonbacterial gastroenteritis. These laboratory assays also enable us to study for the first time prevalence of these agents in different areas of the world and in various age groups. In collaboration with Dr. Peter Echeverria we have previously published several studies on the role of rotavirus in diarrhea among either American soldiers or native populations in South Korea, United States, Taiwan and the Philippines (26,30-34,40-42). More recently with Dr. Echeverria

35. Urasawa,T.,Urasawa,S., and Taniguchi,K.: Sequential passages of human rotavirus in MA-104 cells. Microbiol. Immunol. 25:1025-1035,1981.

36. Provost,P.J. and Hilleman,M.R.: Propagation of human hepatitis A virus in cell culture. Proc.Soc.Exp.Biol.& Med. 160:213-221,1979.

37. Flehmig,B.:Hepatitis A-virus in cell culture. I. Propagation of different hepatitis A-virus isolates in a fetal rhesus monkey kidney cell line (FrhK-4). Med. Microbiol. Immunol. 168:239-248,1980.

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39. Siegl,G.,Frosner,G.G.,Gauss,V. et al: The physiocochemical properties of infectious hepatitis A virions. J.Gen.Virol. 57:331-341,1981.

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41. Echeverria,P.,Blacklow,N.R.,Ho,M.T. et al: Age distribution of antibody to reovirus-like agent in children in Taiwan. J.Ped. 91:960-962,1977.

42. Cukor,G.,Blacklow,N.R.,Capozza,F.E. et al: Persistence of antibodies to rotavirus in human milk. J.Clin.Micro. 93-97, .

we have examined the antibody prevalences to Norwalk virus in the Philippines, Taiwan and the United States (12) and the potential role of Norwalk virus in diarrhea among Peace Corps volunteers who are newly arrived in Thailand (19). Clearly, based on the studies to date, rotavirus and Norwalk virus need to be added to the list of pathogens responsible for diarrhea in different populations, with varying roles for each pathogen in differern population groups.Additional data were collected during the current contract year, which are outlined below.

1. Age Specific Prevalence of Antibody to Rotavirus and Norwalk Virus in a Rural Community in Thailand

In collaboration with Dr. Peter Echeverria of the U.S. Armed Forces Research Institute of Medical Sciences in Bangkok, we have determined age specific antibody prevalence rates for rotavirus and Norwalk virus in the rural farming community of Soongnern, located 240 kilometers northeast of Bangkok. As might be expected, the majority of inhabitants possessed antibody to rotavirus by the age of two years. However, fifty-seven percent of individuals developed Norwalk virus antibody by 5 years of age. This is a rate of antibody positivity reached in United States populations only during adulthood. In contrast, antibody to hepatitis A virus in Soongnern was not acquired until the age of 20 years, suggesting that the epidemiology of infections differs between Norwalk virus and hepatitis A. Evidence that does exist indicates that both viruses are transmitted by the fecal-oral route, and that Norwalk virus can also be spread by a waterborne route. The Soongnern data have been submitted for publication (43).

2. Role of Viral Gastroenteritis Agents in Diarrhea Among Peace Corps Volunteers in Thailand

During the current contract year, Dr. Peter Echeverria carried out a second travelers' diarrhea study among American Peace Corps volunteers during their first five weeks in rural Thailand. The first study, published in June of 1981 (19), revealed major roles for known bacterial pathogens, and infrequent rates of infection with rotavirus and Norwalk virus (one out of 35 individuals infected with each virus over a 10 week period). The second travelers' diarrhea study has provided evidence to support a role for Aeromonas hydrophila as an enteric pathogen which appeared to be inhibited by doxycycline prophylaxis. Virologically, this second study revealed no evidence for infection with rotavirus among 62 Peace Corps volunteers during their first 5 weeks in rural Thailand. Two of the 62 individuals were infected with Norwalk virus. These data have been submitted for publication (44).

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12. Cukor, G.,Blacklow, N.R.,Echeverria, P.,Bedigian,M.K.,Puruggan,H. and Basaca-Sevilla, V.: Comparative study of the acquisition of antibody to Norwalk virus in pediatric populations. Inf. & Immunity 29:822-823,1980.

19. Echeverria,P.,Blacklow,N.R.,Sanford, L.B. and Cukor,G.: Travelers' diarrhea among American Peace Corps volunteers in rural Thailand. J.Inf.Dis. 143:767-771, 1981.

43. Charoenkunl,C. and Yanggratoke,S. Age specific prevalence of antibody to rotavirus, Norwalk virus, hepatitis A and Escherichia coli heat-labile toxin in a rural community in Thailand. Submitted for publication.

44. Echeverria,P.,Sack,R.B.,Blacklow,N.R.,Bodhidatta,P., Rowe,B.,Wauters, G. and McFarland, A. Prophylactic doxycycline for travelers' diarrhea in Thailand: Supportive evidence of Aeromonas hydrophila as an enteric pathogen. Sumitted for publication.

3. Norwalk Virus Outbreaks of Gastroenteritis in Two Nursing Homes

In collaboration with Drs. Wayne Lednar, Jung Park, and Patricia Gulbrandsen (Walter Reed), Drs. Joseph Horman and David Sorley (Maryland Dep't of Health) and Frederick Williams (E.P.A.), we have identified Norwalk virus as the cause of epidemic gastroenteritis in 2 nursing homes in Frederick, Maryland. Illness attack rates were 39 and 34 percent in the 2 institutions, in which both staff members (first) and residents (later) became ill. In this study, Norwalk virus was visualized in a vomitus specimen for the first time in a naturally acquired illness. Epidemiologic and laboratory findings suggested that viral transmission occurred by person to person contact, with stool and vomitus exposures independently contributing to risk of illness. These data have been submitted for publication (45).

4. Diarrhea Due to Norwalk Virus in Texas Families

In collaboration with Drs. Herbert DuPont and Larry Pickering of the University of Texas Health Science Center at Houston, we have assessed the role of Norwalk virus (an important cause of epidemic diarrhea) in endemic diarrhea among families under epidemiological surveillance. Of 28 families under surveillance for 2 years following the birth of a newborn infant,14 families developed outbreaks of diarrhea. Serologic evidence for a Norwalk virus etiology was found in 2 of the 14 outbreaks (14%). This study demonstrates that Norwalk virus is the likely cause of at least a small proportion of family outbreaks of diarrhea. The data have been accepted for publication in the Journal of Infectious Diseases (46).

5. Norwalk Virus and Rotavirus in Travelers' Diarrhea in Mexico

In collaboration with Drs. Herbert DuPont, Bruce Keswick, and John Vollet, we have evaluated the role of Norwalk virus and rotavirus in adult travelers' diarrhea among U.S. students attending a university in Mexico during 1979 and 1980. These student groups were followed for their first 3 weeks in Mexico. Our studies with two separate traveler groups, one year apart, indicate remarkably similar rates each year of infection with Norwalk virus (5 to 6%) and rotavirus (17%). Both viruses play a role in travelers' diarrhea in adults and multiple infections with the two viruses in a single individual over a short time interval may occur. These data were published early in 1982 in The Lancet (18).

PATHOGENESIS STUDIES OF VIRAL GASTROENTERITIS

1. Immunoglobulin M Responses to the Norwalk Virus of Gastroenteritis

During the current contract year, we have performed a study of the presence, characteristics, and potential diagnostic and immunopathologic significance

45. Lednar,W.M., Horman, J.T., Park, J.H., Williams, F.P., Gulbrandsen, P.H., Cukor, G., Sorley, D.L. and Blacklow, N.R.: Outbreaks of Norwalk agent gastroenteritis associated with two nursing homes, 1980-1981, Submitted for Publication.

46. Pickering,L.K.,Blacklow,N.R.,DuPont,H.L. and Cukor,G.: Diarrhea due to Norwalk virus in families. J.Inf.Dis., In Press.

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of the IgM antibody response to Norwalk virus. Heretofore, only total serum antibody responses to Norwalk virus have been examined quantitatively by an RIA blocking test (29). This RIA procedure relies of necessity on carefully selected human clinical materials for its critical reagents because it has not been possible to purify Norwalk antigen from stools sufficiently to permit preparation of useful hyperimmune animal sera. In order to develop an IgM antibody test specific for Norwalk virus, we found it necessary to test fractionated serum in the Norwalk RIA blocking test rather than revise the RIA test to use an anti-human IgM reagent in a direct test. We were not able to use the more practical direct assay probably because of procedural limitations imposed by the relatively low titer of Norwalk antigen shed in stool specimens.

Our work was presented at the National American Society for Microbiology meeting in March of 1982, and has been accepted for publication in Infection and Immunity (47). Briefly our method and results are as follows: IgM, clean of IgG by radial immunodiffusion, has been separated from human serums by sucrose gradient ultracentrifugation. IgM-containing serum fractions are then reacted in the RIA blocking test for antibody to Norwalk virus. The reactivities of these fractions are eliminated by treatment with the reducing agent, dithiothreitol, whereas the reactivities of IgG fractions remain unaffected. We have quantitated titers of IgM antibodies to Norwalk virus in gradient fractions by multiplying the reciprocal of their last positive dilution in the RIA by the quotient obtained by dividing the total IgM concentration (mg/dl) in the whole serum by the total IgM concentration in the pooled IgM fraction prepared from that serum. Expressed in this way, the Norwalk antibody titers of whole serum and gradient fractions are directly comparable.

Eighty-seven serum specimens from 20 human subjects experimentally inoculated one or more times with Norwalk virus were quantitatively examined for virus-specific IgM. The peak IgM response occurred at about 2 weeks after illness, but IgM was detectable in lower titers for up to 21 weeks following infection. The IgM response was seen in volunteers who became ill, whether or not prechallenge total serum antibody was present. On long-term (27 to 42 months) rechallenge, volunteers who were previously ill and had produced IgM antibody again developed illness, and a secondary IgM response which was greater than the first was detected. Inoculated volunteers who did not develop illness, as well as previously ill volunteers on short term rechallenge (4 to 14 weeks), usually failed to generate an IgM response, whether or not an IgG response had occurred. In ill subjects the rise in IgM and IgG occurred concomitantly. Virus-specific IgM is not necessarily indicative of primary infection with Norwalk agent inasmuch as reinfection produces an enhancement of the IGM response. Furthermore, Norwalk-specific IgM responses appear not to be associated with subclinical illness. Additional information about the diagnostic utility of IgM testing for Norwalk virus should be gained from studies of naturally occurring outbreaks of Norwalk virus illness.

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The purpose of this project is to identify, cultivate, and characterize etiologic agents of viral gastroenteritis of man and to study the pathogenesis of infection in order to provide information necessary to attain the ultimate goals of prevention and cure of this common syndrome. Progress achieved during the current year can be summarized as follows. Monoclonal antibody techniques have been utilized successfully to prepare purified antibodies against Norwalk virus with 5 positive clones now prepared. These monoclonal

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20. antibodies are being tested as probes to detect a potential group specific antigen common to the noncytopathic Norwalk-like virus group and to recognize these viruses in nature. In addition,studies are underway to try to cultivate Norwalk virus, using methods that proved successful for the in vitro cultivation of hepatitis A virus and rotavirus.

We have continued studies on the etiology and epidemiology of gastroenteritis with data acquired in 5 study populations: An accelerated development of age specific prevalence of antibody to Norwalk virus has been found in inhabitants of a rural Thailand village. Two of 62 American Peace Corps volunteers were infected with Norwalk virus during their early stay in Thailand. The epidemiology of two nursing home outbreaks of Norwalk virus diarrhea has been studied. This virus has also been shown to be the likely cause of at least a small proportion of endemic family diarrhea in Texas. Finally, both Norwalk virus and rotavirus were shown to play roles in diarrhea among adult travelers to Mexico.

The IgM antibody response to Norwalk virus was assessed in fractionated serum samples from volunteers challenged and rechallenged with the virus. An IgM response occurred in ill subjects, whether or not preexisting total serum antibody was present. The peak response occurred about 2 weeks after illness but IgM was detectable in lower titers for up to 21 weeks after infection. Upon longterm exposure to the virus, previously ill volunteers who had produced IgM antibody once again mounted a secondary IgM response with recurrent illness, which was greater than the first IgM response. Thus, the IgM response is not restricted to primary infection. The potential diagnostic utility of IgM testing for Norwalk virus should be assessed from studies of naturally occurring disease outbreaks.


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